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Bacteriological studies of field soils, II. The effects of continuous cropping and various rotations

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BACTERIOLOGICAL STUDIES OF FIELD SOILS

II. THE EFFECTS OF CONTINUOUS CROPPING AND VARIOUS ROTATIONS

BY P. E. BROWN

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SUMMARY.

From a bacteriological study of soil plots under both continuous cropping and various crop rotations, these general conclusions were drawn:

1. The rotation of crops caused the development of greater numbers of organisms in the soil and of greater ammonifying, nitrifying, and nitrogen-fixing power by the soil, than continuous cropping either to corn or to clover.

2. Greater numbers of organisms, greater ammonifying, nitrifying, and nitrogen-fixing powers were found in a soil under a three year rotation of corn, oats, and clover, than in a soil under a two year rotation of corn and oats, or in a soil under a two-year rotation with clover, cowpeas or oats, turned under as green manure.

3. The use of a green manure in a two-year rotation did not always increase the number of bacteria or the ammonifying, nitrifying, or nitrogen-fixing power of the soil, and it is suggested that the explanation may be sought in the moisture factor or it may be found in the introduction of such large amounts of organic matter.

4. There was an indication that the crop present on the soil was of more importance from the bacterial standpoint than the previous cropping of the soil.

5. The ammonification of dried blood and of cottonseed meal did not always run parallel.

6. The nitrification of dried blood and of ammonium sulfate proceeded almost parallel.

7. Nitrification and ammonification proceeded in the same direction.

8. Evidence is supplied that bacterial activities and crop production are very closely related.

BACTERIOLOGICAL STUDIES OF FIELD SOILS.

II. The Effects of Continuous Cropping and Various Rotations.

BY PERCY EDGAR BROWN.

INTRODUCTION.

In many sections of the country it is a common practice to grow the same crop year after year on the same soil. This is particularly true in the west and middle west, where the soil is still so rich that crop yields have decreased but little as yet because of the practice, and many farmers persist in the wasteful system of continuous cropping to wheat or corn.

Investigators have shown that this continuous cropping of a soil will "wear it out" more or less quickly. They have also been striving for many years to convince the farmers that considerably better crops will be obtained in a few years, and the fertility of the soil conserved for future generations by following a rational system of rotation. This "wearing out" of soils has been attributed to the fact that different crops remove different amounts of plant food constituents. One element may be used in much larger quantities than the other constituents by a certain crop, and very soon, then, that element will become the limiting factor of growth, and the crop will fail, although the other necessary constituents may be present in ample amounts. By growing this crop alternately with others which do not remove so much of the same element, opportunity is given for the soil to regain its normal stock of that particular constituent, and consequently the fertility or crop-producing power of the soil is not diminished.

Various rotations are advocated for different conditions, some being of general value and some adapted to local conditions. It is commonly conceded now, however, that a good rotation should include clover, or some other legume in order not only to maintain the nitrogen content of the soil which in so many cases is the governing factor for the growth of plants, but also to supply humus or organic matter. Furthermore, in soils where humus is very deficient, it is a common practice, following a small grain crop, to plow under a legume for green manure. In general, it may be said that a three year rotation of corn, oats, and clover, or a four year rotation of corn, corn, oats, and clover, are the two systems generally used to the best advantage in this section of the country.

Another explanation has been offered by the Bureau of Soils[1] to account for the failure of a crop where rotation is not practiced, and that is that certain complex organic compounds called "toxic substances" are excreted in the growth of plants and that they are injurious to the same plants when grown the next year, but that they may be harmless to other crops or may be neutralized by the excretions of other crops. It has been stated that the injurious action of such toxic substances may be obviated, therefore, by the proper rotation of crops, different systems of course being advocated for different conditions. They have even gone so far as to say that the beneficial action of fertilizers lies not so much in the supplying of plant food constitutents as in the neutralization of these toxic substances.

Several organic compounds have been isolated by the investigators mentioned, and have been shown to be injurious when added in small amounts to water cultures of certain plants. These results, however, have seemingly been too broadly interpreted, for it is believed that the results of water culture tests should hardly be considered applicable to soil conditions as in the latter case many other factors may influence the effects of such toxic substances on plants. In general these views of the Bureau have seemed too extreme to most scientists and have not commonly been accepted, and in some directions they have been strongly combated, although no experimental proof has yet been advanced to disprove them.

It is certain, however, whether it is due to toxic substances or to unequal utilization of the different plant food constituents that the continuous growing of one crop is bad practice, and leads not only to a decrease in crop yields, but also brings about a more or less rapid exhaustion of the soil.

Now it is undoubtedly the case that plants in their growth excrete certain organic substances and in the decay of plant residues, certain other complex compounds are produced, whose nature depends on the character of the crop. While it is still a question whether these substances accumulate in sufficient quantities to influence the growth of the same crop the succeeding year, and whether they may be neutralized or rendered harmless by the growth of some other crop, it is probably true that the effect of such substances on the bacterial flora of the soil is very great.

No work has been done along this line and investigations are now in progress under the writer's direction at the Iowa Agricultural Experiment Station to determine the effects of certain organic substances on some important bacterial species in the soil.

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[1]Bul. 22, Bureau of Soils.

It seemed to the writer that possibly the cause for the reduction of crop yields and the exhaustion of soils under continuous cropping, was bacterial rather than chemical, or perhaps bacterial **and** chemical in nature. In other words, it is conceivable that the depletion of bacterial food in the soil might depress the activities of the organisms to such an extent that plant food would not be produced in sufficient amounts to support optimum crop growth. Furthermore, it is likewise conceivable that the presence of certain organic substances in the soil might reduce bacterial activities so that the succeeding crop would not be properly supplied with food.

The effects of continuous cropping and of different rotations on the bacterial flora of soils have as yet never been studied and consequently the first step in this investigation must necessarily consist in the solution of these problems—whether continuous cropping reduces bacterial activities and whether different methods of rotation encourage or discourage bacterial growth. If these facts can be proved, then the next step will be the determination of the cause of such variations in bacterial activities.

The investigations reported in this bulletin, therefore, constitute a contribution toward the solution of the first part of this problem, and while the results of one year's investigations must not be considered conclusive, the indications are that bacteria are profoundly affected by different methods of cropping and consequently they may be held largely responsible for the effects on crops of continuous cropping and for the varying values of different rotations.

The peculiar character of field conditions and the various factors which must be considered in a study of bacteria in field soils have been pointed out in the introduction to this series of bulletins and need not be discussed here. It may be said, however, that great care has been exercised in drawing conclusions from these field results and all factors and conditions of the experiment have been carefully considered and generalizations have not been attempted.

It is intended to continue the work in the future and it remains, therefore, for the combined results of several years' work to establish or disprove the conclusions which are suggested.

It should be noted here also that the work is intended to lend additional information to the subject of the relations between bacterial activities as tested in he laboratory and crop production, and to this end the crop yields from the plots employed in the investigation have been included and will be studied in this and in future work in connection with the bacterial studies.

HISTORICAL.

As has been stated, no work has been done in the past to show the effects of different rotations on bacterial activities in the soil. Consequently the only references which it may be of value to mention here are those which deal with studies of the effects of different crops on the numbers and activities of certain groups of bacteria. It will be understood that these investigations are related to the present work only in so far as the differences brought about in the bacterial flora of soils under different rotations are due to the effects of the growth of certain crops. The length of the rotation, that is, the time which elapses between the growth of the same crop twice on the same soil, will undoubtedly play an important part in determining its effect on bacterial activities. In consequence, therefore, of the fact that it is the growth of different crops which determines the effects of rotations on bacteria, it will be interesting to note the following experiments.

Caron[1] found the greatest number of organisms in fallow soils, large counts being obtained from soils under forage crops and the lowest numbers from soils under grain crops. Burri's[2] results showed the same number of organisms present in a garden soil as in a soil from a clover field and over twice as many in a soil under rye. The figures which he obtained from meadow and forest soils were exceedingly variable and show the great differences which occur in such soils. Stoklasa and Ernest[3] found that to a depth of 30 cm., soil from under clover contained twice as many organisms as that from under sugar beets and six or seven times as many as that from under barley.

The early work of Bretschneider[4] showed different amounts of ammonia present in soils under turnips, vetch and oats, and in uncropped soils. While the amounts present were the same in April, at succeeding dates variations appeared; but they were so irregular that any conclusions would be difficult. The varying moisture conditions probably played an important part in these results and possibly were of more importance than the different crops.

Voorhees, Lipman, and Brown[5] found decomposition slower under oats and clover than in bare soils. These results were obtained by the gelatin solution method for testing the ammonifying power of soils and were therefore rather irregular and inconclusive.

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[1]Landw. Vers. Stat. 45, 1895, p. 403.
[2]Schweiz. landw. Zentralbl. 20, 1901, p. 215.
[3]Centbl. f. Bakt. 2 (etc) Abt. 14, 1905, p. 725.
[4]Mitt d. landw. Zentralver. f. Schleisen 14, 1865, p. 121.
[5]Bul. 210. (1907) N. J. Agri. Expt. Station, p. 38.

Bretschneider* also determined the nitrates present under various crops and found some very large differences, the largest amounts of nitrates were found in most cases under turnips and in uncropped plots, being much larger than the amounts found under vetch or oats.

King and Whitson[2] found differences in the nitrates produced under the same crop in different plots and under different crops, and while the differences in some cases were quite considerable, conclusions would hardly be permissible.

Deherain[3] claimed that if the soil moisture was not lacking and if the loss of nitrate nitrogen in the drainage and the nitrogen in the crop were added similar nitrification would be found in fallow and in cropped lands. This statement, however, has been largely opposed and in the question of nitrification under legumes which has received considerable attention, experiments have shown that sometimes more and sometimes less nitrate nitrogen may be found than under grain crops.[1]

In studying nitrogen fixation Berthelot[4] found less rapid fixation of nitrogen by free living organisms in cropped than in uncropped soils, and thought that it might be due in many cases to moisture conditions. It has been shown, however, that if moisture conditions are maintained at the optimum, the nitrogen fixing bacteria may be very active under a very heavy crop.[5]

THE PLOTS EMPLOYED.

The plots chosen for this work were selected from a plot experiment of the Agronomy Section. That experiment included 14 plots, one-tenth of an acre in size, located on a typical Wisconsin drift soil, the particular type being known as Marshall loam. The land is somewhat rolling but not

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*Mitt. d. landw. Zentralver. f. Schleisen 14, 1865, p. 121.

[1]Lawes, Gilbert & Warington, Journ, R. Agric. Soc. 19, 1883; ref. Jahres d. Agric. Chem. 26, p. 42.
Wollny-Vierteljahresschr. d. bayr. Landw. Rats. 1897, 3 & 4, ref. Zentralbl. f. Agric. Chem. 29, 1900, p. 509.
King & Whitson, Rpt. Wis. Exp. Sta. 1900, p. 204· ref. Expt. Sta. Rec. 13 p. 24.
Montanari, Staz. sperim. agrar. ital. 41, 1908, p. 209; ref. Expt. Sta. Rec. 20, p. 716.
Lawes & Gilbert, Jahresber. d. Agric. Chem. 26, 1883, p. 38.
Beeson, Journ. Am. Chem. Soc. 20, 1898, p. 793.
Hanamann, Zeitschr, f. d. Landw. Wesen. in Österreich 4 (1901) p. 34.
Troubetzkoy, Result. d. travaux de la Stat. Exp. de Ploty 1895-1904, p. 46.

[2]Bul. 93, Wisconsin Agri. Expt. Station.

[3]Compt. Rend. 125 (1897) p. 209; Ann. Agron. 23 (1897) p. 241.

[4]Compt. Rend. 104 (1887) p. 625.

[5]Liebscher, Journ. f. Landw. 41 (1893) p. 156.
Kowerski, Der Weisse Senf. als Stickstoffvermehrer etc., Diss phil. Halle 1895.
Pfeiffer, Frank, Mitt. Landw. Inst. Breslau 4 (1909) p. 758.

enough to affect seriously its value for experimental purposes.

Prior to 1907 the land was under a four-year rotation and in that year the differentiation of the plots according to the following plan was begun:

Plot	Treatment
601	Continuous corn.
602, 603	2-year rotation, corn and oats.
604, 605, 606	3-year rotation, corn, oats, and clover.
607, 608	2-year rotation, corn and oats, clover plowed under after the oats.
609, 610	2-year rotation, corn and oats, cowpeas plowed under after the oats.
901, 902	2-year rotation, corn and oats, rye plowed under after the oats.
903	Continuous clover.
904	4-year rotation, corn, corn, oats, and clover. (Check plot of another series.)

This experiment, which was carried on during 1911, the fourth year of the special treatment, included the study of eight of these plots: 601, 602, 604, 607, 609, 901, 903 and 904. The first six of these were chosen because they were all in corn and it was thought that the effects of the previous treatment could be studied more satisfactorily where the influence of the crop growing at the time of the experiment was presumably the same for all the plots. Plot 903, of necessity, represented conditions somewhat different from the first six and the results from 904 are not strictly comparable with the others, as it was in clover in 1911, but they are included for the sake of comparison and because they bring out some interesting facts. It is purposed in future work to study the entire series of plots and thus perhaps throw some light on the relative effects of **previous** crops and **present** crops on the bacterial activities in the soils. It must be kept in mind in the subsequent discussions in this work, therefore, that plots 903 and 904 were in clover and the other six plots were in corn and the differences which appear in the results must not be attributed entirely to the system of cropping, they may be due in large part to the differences in treatment, e. g., in the cultivation for the clover and the corn. These differences in treatment would bring about variations in aeration, moisture, and temperature conditions in the soil which would probably affect bacterial activities quite materially.

The plots were managed with the usual precautions used in such experiments, to avoid the transference of soil, and the crop was harvested as usual and the yields per acre from the various plots were calculated. The results for 1911 will be given later in this bulletin and comparisons made with the results of the bacteriological tests.

The bacteriological examination of the soils consisted in determinations of the numbers of organisims present in the soils at the various samplings, by counting the colonies developing on "modified synthetic" agar plates, and in determinations of the ammonifying, nitrifying, and nitrogen fixing powers of the soils by the beaker method.

METHODS OF SAMPLING.

The method of sampling was the same as described in the first bulletin of this series[1]:

"The surface two to three inches of soil were removed by means of a sterile trowel from an area about twenty inches square, the soil in that area was thoroughly stirred and mixed to a depth of four to six inches, and the samples were drawn and taken to the laboratories in sterile glass jars." In this work also four samplings were made, one in June, one in July, one in September, and one in October, and the same care was observed here that succeeding samples from the same plot should be taken from a different part of the plot. In almost all cases, as will be seen later, the same differences in the soils were brought out at the different samplings, those instances in which this was not the case being easily accounted for through the variation in some factor such as moisture, etc. The assumption seems justifiable here also, therefore, "that the local differences in the separate plots have been largely eliminated by the methods employed or at least have been obliterated by the action of the special treatment under consideration."[2]

THE QUANTITATIVE DETERMINATIONS.

The results of the quantitative determinations are given in Table I, and Table II contains the moisture determinations. The "modified synthetic" agar plate method was employed in this work and the usual dilutions were prepared from which quantities representing one-twenty-thousandth and one two-hundred-thousandth of a gram of soil were plated. Duplicate plates were prepared and the results which are given are the averages of the counts made on these plates at the end of three days' incubation at 20° C.

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[1]Research Bul. No. 4, Iowa Agric. Exp. Sta.
[2]Ibid.

Considering the results as a whole, several facts appear quite evident. The two-year rotation increased the number of bacteria in the soil beyond that of the continuous corn plot and the three year rotation caused a still further increase. These gains were quite definite and appeared at every sampling, notwithstanding in some cases differences in moisture conditions which would tend to bring the numbers closer together. The increases were the greatest where the moisture content was more nearly uniform, e. g., September 16, and were more prominent also at those dates when the moisture content of all the soils was greatest. The effects of the clover, cowpeas, and rye turned under as green manure in the two year rotation were somewhat irregular and definite conclusions may not be drawn.

TABLE I.

THE QUANTITATIVE DETERMINATIONS.

(Bacteria per gram of air-dry soil.)

Plot No.	I. June 26.	II. July 8.	III. Sept. 16.	IV. Oct. 25.
601	2,186,000	2,209,000	2,100,000	1,858,000
602	2,700,000	2,681,000	2,550,000	2,500,000
604	3,210,000	3,209,000	3,450,000	3,329,000
607	2,538,000	2,488,000	3,219,000	3,058,000
609	2,968,000	2,793,000	2,716,000	2,391,000
901	2,658,000	2,574,000	2,536,000	2,343,000
903	1,195,000	1,118,000	1,756,000	1,619,000
904	2,234,000	2,537,000	2,360,000	2,744,000

TABLE II.

MOISTURE IN SAMPLES.

Plot No.	June 26. I. Percent	July 8. II. Percent	Sept. 16. III. Percent	Oct. 25. IV. Percent
601	19.50	14.00	20.00	15.00
602	20.00	10.50	20.00	16.00
604	21.50	11.50	20.00	15.50
607	16.50	10.00	18.00	16.50
609	20.50	10.50	20.50	15.00
901	18.00	13.00	18.00	15.50
903	18.00	7.00	18.00	16.00
904	10.50	7.00	15.50	14.00

At the first two samplings the clover seemed to have no effect, in fact slightly smaller numbers than in the two year rotation plot seeming to indicate possibly a depression, but at those samplings the moisture content of the soil was very low and consequently that fact would probably partly explain the differences. At the last two samplings the clover showed an increased number of bacteria over the two year rotation, but not as great as that accomplished by the three year rotation. The cowpeas turned under in the two year rotation gave greater numbers of bacteria than the ordinary two year rotation at the first three samplings, but at the last date a slightly smaller number was observed, due in part probably to a somewhat lower moisture content. The numbers found were in every case lower than those obtained under the three year rotation. The rye as a green manure in every case gave smaller numbers of bacteria than the ordinary two-year rotation, the differences in moisture content being insufficient in most cases to account for the smaller numbers. The continuous cropping to clover in every case reduced the number of organisms, this reduction appearing very prominently even when no differences in moisture content were recorded. The four-year rotation of corn, corn, oats, and clover, in only one case showed a gain over the two-year rotation, but the results obtained here were probably due to the difference in the crops grown during the season of the experiment. The difference in cultivation and consequent aeration and moisture would probably account in part for the lower numbers.

THE EFFECT OF DROUGHT.

Comparing the results at the different samplings in most cases they were rather uniform; with some exceptions the same differences appeared in each case, being more prominent at those dates when the moisture content of the soils was the greatest. There were fewer numbers recorded at the second than at the first date in practically every case, and this corresponded to lower moisture content in the soils. Between the second and third sampling the weather was very dry and probably the bacterial content of the soils went very low. The numbers had increased, however, before the third sampling and the differences which appeared at this date, therefore, were somewhat greater and in the case of the plots where green manuring was practiced, not exactly the same as at the previous samplings.

It is believed that drought materially alters the species relationships existing in the soil and such seemed here to be the case, the greater relative numbers of bacteria in the green manured plots in the two cases following the drought giving evidences of multiplication of bacteria along somewhat different lines, probably induced by the green manure. At the fourth sampling a decrease in bacteria in all the plots was observed, due primarily, undoubtedly, to the decrease in moisture content which occurred following the third date. The differences between the plots, however, were practically identical with those observed at the third date. Consequently the variations in the results which appear at the four samplings may be attributed to the effect of the drought which occurred beween the second and third sampling, on the bacterial flora of the soil, altering the species relationships.

With regard to the cause of these differences in the bacterial flora of soils under different rotations, we can do no more than theorize. It will be remembered that in the introduction to this bulletin two possibilities were suggested as explanations for the benefits of rotations on the crop yields. Now either of these theories may be fitted to the present results.

TESTING THEORIES BY RESULTS.

In the first place, perhaps the continuous growth of a crop such as corn or clover, removes from the soil a disproportionate amount of one plant food constituent and soon the soil becomes deficient in that one element, although all the others may be present in large amounts. If this is the case, then some effect of such a depletion of the store of one constituent on bacteria would be expected. That is, certain bacterial activities would be just as certain to be restricted by the absence of some food constituent as would certain crop yields. Consequently, the theory fits the results from the first three plots quite satisfactorily for the continuous cropping reduced the bacterial flora of the soil below any method of rotation. Furthermore, the three year rotation where clover was grown gave greater numbers of organisms than the two year rotation where only corn and oats were grown. This increase was possibly due to the nitrogen and organic matter supplied by the clover crop.

Looking over the results where green manure was applied in the two-year rotation, it seems, however, that some other factor must have been at work for we would naturally expect that turning under an entire crop, e. g. of clover, would have more effect than the cropping to that plant because of the addition of a larger amount of plant food constituents, but such was not the case. Now green manures require the presence of large amounts of water to accomplish their decomposition; and if enough water is not present, there will be no observed increase and may even be a decrease in crop production due to the removal of water from the use of the plants. It is conceivable, therefore, that a green manure may remove enough water from the plants to reduce the crop yield and still have insufficient to encourage bacterial activities very materially; and in fact it may even reduce bacterial action by not leaving enough water for the bacteria to perform their normal functions to the best advantage. This moisture factor would probably be of sufficient importance to account for the figures at some of the samplings but it is inadequate to explain all, and some other factor must, therefore, be held accountable.

APPLYING THE "TOXIC" THEORY.

Turning to the second theory suggested to account for the effects of rotations, namely, the "toxic" theory, it will be seen that the benefits of the two and three year rotations over the continuous cropping may be explained on the basis of the accumulation of toxic substances where the same crop is grown year after year and the reduction by these substances of bacteria in the soils. The alternation of a crop with one or two others, however, tends to nullify the effects of these toxic substances from these other crops; or possibly through a reduction in the amount of any one toxic substance it allows opportunity for the bacteria to dispose of them in a normal way without the organisms being reduced in numbers or efficiency.

It is possible that the disposal of such toxic substances may be accomplished by the action of bacteria; certain specific groups may decompose them and take out what they need for food, or the products of the bacterial decomposition of some other materials may act on the substances in a purely chemical way and destroy them or change them to a harmless form or to a form which may actually be available for bacterial food. When a green manure is used on a soil a large amount of organic matter is introduced and it is possible that this organic matter may prevent the disposal of the toxic substances by inhibiting the action of the bacteria which usually accomplish their destruction, or by neutralizing the compounds which ordinarily are utilized in the neutralization of the toxic substances. At any rate, it would seem from these results that the green manure was less efficient in increasing the numbers of organisms and, as will be seen later, in increasing the crop, than the 3 year rotation where a longer period was allowed between two succeeding crops of the same plant and there was not the interference of a large amount of organic matter. It must be remembered in this connection, however, that the season of the experiment was unusually dry and the differences noted might not be borne out in a normal season.

But whatever the explanation of the effects of the different rotations, it is evident that the numbers of bacteria in the soil were materially affected by the use of different rotations; in other words, the growth of different crops in previous years affected appreciably the bacteria in the soil during the season when the same crop was being grown.

CONCLUSIONS.

The conclusions which may be drawn from these quantitative determinations, are therefore:

1. The rotation of crops caused the development of greater numbers of organisms in the soil than continuous cropping either to corn or to clover.

2. Greater numbers of organisms were found in a soil under a three year rotation of corn, oats, and clover, than in that under a two year rotation of corn and oats, or in those under two year rotations in which clover, cowpeas, and rye were used as green manures.

3. The use of such a green manure in the two year rotation did not always increase the number of bacteria in the soil. It is suggested that possibly the moisture factor or the introduction of organic matter in such large amounts may supply the explanation.

4. There was an indication that the influence of the crop present on the soil was greater than the influence of the previous cropping on the bacteria in the soil and this was probably due to the difference not only in the crop but in the treatment of the soil and preparation for the crop.

THE AMMONIFICATION EXPERIMENTS.

The determination of the ammonifying, nitrifying, and nitrogen fixing powers of the soils were made, as has been stated, by the beaker method. This has been described in detail in previous work[1] and need not be discussed here as the methods employed were exactly the same as those employed in the experiments cited.

The results of the ammonification tests are given in Tables III, IV, V, and VI, and the summarized results for the ammonification of dried blood and of cottonseed meal at the four samplings may be found in Tables VII and VIII.

Considering first the ammonification of dried blood, we note at every sampling a gradually increasing ammonifying power from the continuous corn plot to the three year rotation plot, the differences appearing the most prominently at the third sampling where 133.43 mgs. N. were formed as ammonia by the three year rotation plot against 117.86 mgs. N. for the two year rotation, and 108.76 mgs. N. for the continuous corn plot.

At the first two samplings the ammonifying power of the soil from the plot under the two year rotation with clover

[1]Brown, Research Bul. No. 2, Iowa Agric. Expt. Sta. 1911.

turned under was less than that of the soil under the ordinary two year rotation, but at these dates the moisture content of the soil was small, much less than that of the other plots, this being probably the cause, at least in part, of the lower ammonifying power. At the two latter samplings the ammonifying power of the soil from this plot was much greater than that of the soil under the ordinary two year rotation, but still slightly smaller than that of the soil under the three year rotation.

The ammonifying power of the soil under the two year rotation with cowpeas as a green manure was less than that of the soil under the three year rotation at every sampling, but larger than that of the soil under the two year rotation, the differences in this latter case being very small.

TABLE III.

AMMONIFICATION EXPERIMENTS I.

Plot No.	Lab. No.	Addition	Average mgs. N.	Ammonia mgs. N.
601	61	5 gms. D. B.	168.27	
	62	5 gms. D. B.	173.96	171.11
	63	5 gms. C. S. M.	140.75	
	64	5 gms. C. S. M.	143.28	142.01
602	65	5 gms. D. B.	179.02	
	66	5 gms. D. B.	177.12	178.07
	67	5 gms. C. S. M.	143.28	
	68	5 gms. C. S. M.	145.81	144.54
604	69	5 gms. D. B.	190.72	
	70	5 gms. D. B.	186.93	188.82
	71	5 gms. C. S. M.	148.34	
	72	5 gms. C. S. M.	154.03	151.18
607	73	5 gms. D. B.	178.07	
	74	5 gms. D. B.	172.38	175.22
	75	5 gms. C. S. M.	147.07	
	76	5 gms. C. S. M.	143.91	145.49
609	77	5 gms. D. B.	178.70	
	78	5 gms. D. B.	181.23	179.96
	79	5 gms. C. S. M.	148.66	
	80	5 gms. C. S. M.	148.34	148.50
901	81	5 gms. D. B.	177.12	
	82	5 gms. D. B.	172.38	174.75
	83	5 gms. C. S. M.	142.96	
	84	5 gms. C. S. M.	145.18	144.07
903	85	5 gms. D. B.	151.82	
	86	5 gms. D. B.	149.60	150.71
	87	5 gms. C. S. M.	126.52	
	88	5 gms. C. S. M.	134.11	130.31
904	89	5 gms. D. B.	175.54	
	90	5 gms. D. B.	175.54	175.54
	91	5 gms. C. S. M.	147.07	
	92	5 gms. C. S. M.	148.66	147.86

At every sampling but the second, the ammonifying power of the soil under the two year rotation with rye turned under, was less than that of the soil under the regular two year rotation, and at the second date the difference was so small that it should hardly be considered.

The ammonifying power of the continuous clover plot was very low in every case, being lower than that of the continuous corn plot.

The results from the four year rotation plots were very irregular. At the first and third samplings, the ammonifying power of the soil was less than that of the soil under the two year rotation, but at the second and fourth dates it was more than that of that soil but less than that of the soil under the three year rotation. In some cases the moisture content of the soil might be held accountable for some of the differences manifested, but generally the differences appeared in spite of the moisture conditions.

TABLE IV.

AMMONIFICATION EXPERIMENTS II.

Plot No.	Lab. No.	Addition	Ammonia mgs. N.	Average mgs. N.
601	361	5 gms. D. B.	218.12	
	362	5 gms. D. B.	223.36	220.74
	363	5 gms. C. S. M.	162.28	
	364	5 gms. C. S. M.	164.37	163.32
602	365	5 gms. D. B.	231.73	
	366	5 gms. D. B.	231.03	231.38
	367	5 gms. C. S. M.	169.96	
	368	5 gms. C. S. M.	167.52	168.74
604	369	5 gms. D. B.	244.30	
	370	5 gms. D. B.	242.90	243.60
	371	5 gms. C. S. M.	176.24	
	372	5 gms. C. S. M.	179.38	177.81
607	373	5 gms. D. B.	231.38	
	374	5 gms. D. B.	227.89	229.63
	375	5 gms. C. S. M.	171.70	
	376	5 gms. C. S. M.	164.72	168.21
609	377	5 gms. D. B.	238.01	
	378	5 gms. D. B.	239.06	238.53
	379	5 gms. C. S. M.	168.21	
	380	5 gms. C. S. M.	173.80	171.00
901	381	5 gms. D. B.	229.99	
903	382	5 gms. D. B.	234.17	232.08
	383	5 gms. C. S. M.	167.86	
	384	5 gms. C. S. M.	164.03	165.94
	385	5 gms. D. B.	207.65	
	386	5 gms. D. B.	213.58	210.61
	387	5 gms. C. S. M.	158.09	
	388	5 gms. C. S. M.	158.79	158.44
	389	5 gms. D. B.	238.36	
	390	5 gms. D. B.	233.83	236.09
	391	5 gms. C. S. M.	170.66	
904	392	5 gms. C. S. M.	174.15	172.40

Looking over the results with cottonseed meal, we find the differences which are shown are in most cases similar to those obtained with the dried blood.

The two and three year rotations in every case increased the ammonifying power of the soil over that of the soil under continuous corn. The results obtained from the plots where green manures were used were again very irregular. They did not all check at the various samplings, nor did they all agree with the dried blood results. Where clover was turned under, greater ammonifying power was found than in the soil under the regular two-year rotation, at three samplings and at the second date practically identical amounts were obtained on the two plots. At the third sampling, a higher ammonifying power was found in the clover plot than in the three year rotation plot, this being the only case where greater bacterial activities

TABLE V.

AMMONIFICATION EXPERIMENTS III.

Plot No.	Lab. No.	Addition	Ammonia mgs. N.	Average mgs. N.
601	661	5 gms. D. B.	107.93	
	662	5 gms. D. B.	109.59	108.76
	663	5 gms. C. S. M.	102.30	
	664	5 gms. C. S. M.	101.97	102.13
602	665	5 gms. D. B.	119.85	
	666	5 gms. D. B.	115.88	117.86
	667	5 gms. C. S. M.	108.93	
	668	5 gms. C. S. M.	111.25	110.09
604	669	5 gms. D. B.	135.75	
	670	5 gms. D. B.	131.11	133.43
	671	5 gms. C. S. M.	120.18	
	672	5 gms. C. S. M.	120.18	120.18
607	673	5 gms. D. B.	131.11	
	674	5 gms. D. B.	128.46	129.78
	675	5 gms. C. S. M.	128.79	
	676	5 gms. C. S. M.	133.43	131.11
609	677	5 gms. D. B.	117.87	
	678	5 gms. D. B.	119.19	118.53
	679	5 gms. C. S. M.	105.62	
	680	5 gms. C. S. M.	105.95	105.78
901	681	5 gms. D. B.	115.88	
	682	5 gms. D. B.	118.20	117.04
	683	5 gms. C. S. M.	114.89	
	684	5 gms. C. S. M.	110.58	112.73
903	685	5 gms. D. B.	100.98	
	686	5 gms. D. B.	99.33	100.15
	687	5 gms. C. S. M.	93.37	
	688	5 gms. C. S. M.	99.33	96.35
904	689	5 gms. D. B.	107.27	
	690	5 gms. D. B.	109.26	108.26
	691	5 gms. C. S. M.	117.20	
	692	5 gms. C. S. M.	115.88	116.54

TABLE VI.

AMMONIFICATION EXPERIMENTS IV.

Plot No.	Lab. No.	Addition	Ammonia mgs. N.	Average mgs. N.
601	801	5 gms. D. B.	109.26	
	802	5 gms. D. B.	111.91	110.58
	803	5 gms. C. S. M.	113.23	
	804	5 gms. C. S. M.	108.93	111.08
602	805	5 gms. D. B.	117.20	
	806	5 gms. D. B.	115.88	116.54
	807	5 gms. C. S. M.	121.84	
	808	5 gms. C. S. M.	122.50	122.17
604	809	5 gms. D. B.	132.44	
	810	5 gms. D. B.	129.79	131.11
	811	5 gms. C. S. M.	128.13	
	812	5 gms. C. S. M.	125.15	126.64
607	813	5 gms. D. B.	125.15	
	814	5 gms. D. B.	124.49	124.82
	815	5 gms. C. S. M.	124.16	
	816	5 gms. C. S. M.	122.83	123.49
609	817	5 gms. D. B.	116.54	
	818	5 gms. D. B.	117.15	116.84
	819	5 gms. C. S. M.	120.85	
	820	5 gms. C. S. M.	117.20	119.02
901	821	5 gms. D. B.	113.89	
	822	5 gms. D. B.	115.88	114.88
	823	5 gms. C. S. M.	116.21	
	824	5 gms. C. S. M.	114.89	115.55
903	825	5 gms. D. B.	99.33	
	826	5 gms. D. B.	104.29	101.81
	827	5 gms. C. S. M.	104.29	105.12
	828	5 gms. C. S. M.	105.95	
904	829	5 gms. D. B.	123.16	
	830	5 gms. D. B.	122.50	122.83
	831	5 gms. C. S. M.	122.50	
	832	5 gms. C. S. M.	119.19	120.84

TABLE VII.

THE AMMONIFICATION OF DRIED BLOOD.

Plot No.	June 26. I. mgs. N.	July 8. II. mgs. N.	Sept. 16 III. mgs. N.	Oct. 25. IV. mgs. N.
601	171.11	220.74	108.76	110.58
602	178.07	231.38	117.86	116.54
604	188.82	243.60	133.43	131.11
607	175.22	229.63	129.78	124.82
609	179.96	238.53	118.53	116.84
901	174.75	232.08	117.04	114.88
903	150.71	210.61	100.15	101.81
904	175.54	236.09	108.26	122.83

were found in the clover plot than in the three-year rotation plot. At the first two samplings the use of cowpeas seemed to increase the ammonifying power of the soil over the ordinary two-year rotation, but at the last two dates the opposite was the case. This difference in the results at the four samplings might be attributed largely to the drought and the changes in bacterial flora produced thereby. With rye, in every case but one (where the amounts were practically the same) smaller ammonifying power was shown than in the soil under the regular two-year rotation. The effect of continuous cropping to clover was to lower the ammonifying power of the soils, very considerably, due both to the crop grown and to the method of treatment of the soil in preparation for the crop and to absence of cultivation of the soil. The four-year rotation in three cases showed a greater ammonifying power than the ordinary two-year rotation and at the last sampling where a slightly smaller power was found, the difference was too small to be of significance.

Comparing the results secured where dried blood and where cottonseed meal were employed, we find that in some cases the differences in ammonifying power which they show were about the same, but when green manures were used the ammonification of the two materials did not proceed similarly. It has been noted previously that the ammonification of these materials does not always proceed similarly and the difference has been attributed to the difference in the carbon-nitrogen ratio in the two materials. It is interesting to note in this connection then, that in this experiment, the ammonification of the two materials proceeded parallel except where green manure was used and in this case the introduction of a large quantity of organic matter evidently affected the carbon-nitrogen ratio in the soil, and consequently altered the species relationships existing there, and the ammonifying power of the soil as tested

TABLE VIII.

THE AMMONIFICATION OF COTTONSEED MEAL.

Plot No.	June 26. I. mgs. N.	July 8. II. mgs. N.	Sept 16. III. mgs. N.	Oct. 25. IV. mgs. N.
601	142.01	163.32	102.13	111.08
602	144.54	168.74	110.09	122.17
604	151.18	177.81	120.18	126.64
607	145.49	168.21	131.11	123.49
609	148.50	171.00	105.78	119.02
901	144.07	165.94	112.73	115.55
903	130.31	158.44	96.35	105.12
904	147.86	172.40	116.54	120.84

gave evidence of these differences by the different action on dried blood and on cottonseed meal.

Considering the results of the ammonification tests as a whole, some facts appear very distinctly while others are obscured by various factors not controlled.

The beneficial effect of a two-year and three-year rotation in increasing the ammonifying power of the soil over that of the soil under continuous corn are clearly shown, and the depression of the ammonifying power of the soil under continuous clover even below that of the continuous corn plot is also quite evident. As has been stated, the results from the plots where green manure was used were too irregular to permit of any definite conclusions.

In most cases the power of these soils to produce ammonia was less than that of the three-year rotation plot, but it was not always greater than that of the two-year rotation plot, in fact in the case of the rye plot, in almost every case, it was less than the power of the two-year rotation plot. It seems therefore, that the introduction of large quantities of organic matter interfered considerably with the ammonifying species in the soil but just how or why that interference was brought about is still a matter of conjecture.

AGREEMENT BETWEEN DETERMINATIONS.

Remarkable agreement between these results and those of the quantitative determinations are also shown. Increased total numbers of organisms and increased ammonifying power appear coincident, and the theories which are advanced under the discussion of the quantitative determinations will fit very well the ammonification results. That is, it may be that the continuous growing of one crop reduces the ammonifying power of the soil because of the over-utilization of some particular element of food which is required by a prominent ammonifying species, or because of the accumulation of some substances produced in the growth of the particular crop which inhibit the growth of some ammonifiers. The alternate growing of the particular crop with some other or with other crops may check the utilization of the particular element or the substances which restrict the growth of the ammonifiers may be neutralized or destroyed. It is evident here, too, that the introduction of large quantities of green manure did not increase the ammonifying power of the soil as much as a normal three-year rotation, and while, as was suggested, this was probably due in large part to the moisture conditions, there is the possibility that there was some effect on the bacterial flora of the soil by the organic matter in the crop, or possibly there was not the same action on the so-called toxic substances as was brought about by the normal growth of the crop. There is an indication from these results also that the ammonifying power of the soil was affected more by the crop growing on the soil than by the crops previously grown, and this of course was largely due to the difference in treatment of the soils. The differences observed in this case should by no means be interpreted for all crops, as the difference in treatment for clover and corn is very great and with other crops where the difference in treatment is not so great the facts mentioned would perhaps not be observed.

CONCLUSIONS OF AMMONIFICATION TESTS.

The result of the ammonification tests as a whole may be given, therefore, as follows:

1. The ammonifying power of a soil where a three-year rotation of corn, oats, and clover, was practiced, was greater than that of a soil under a continuous crop of corn or clover.
2. The ammonifying power of a soil where a three-year rotation of corn, oats, and clover was practiced, was greater than that of a soil under a regular two-year rotation and greater than that of a soil under a two-year rotation with clover, cowpeas, or rye used as a green manure.
3. The use of a green manure crop such as clover, cowpeas, or rye, in a two-year rotation of corn and oats, did not always increase the ammonifying power of the soil, possibly due to the limiting action of moisture conditions or due to the effects of the organic matter which was introduced.
4. The effects of previous treatment on the ammonifying power of the soil under the four year rotation was probably obscured by the growth of the clover crop which diminished bacterial activities through absence of cultivation and consequently diminished aeration and moisture conditions.
5. The ammonification of dried blood and of cottonseed meal did not always run parallel. Large amounts of organic matter introduced into the soil may so alter species relationships that the results obtained with these two substances will not be comparable.

THE NITRIFICATION EXPERIMENTS.

The results of the nitrification experiments are given in Tables IX, X, XI, and XII, and Tables XIII, and XIV show the summarized results for the nitrification of ammonium sulfate and of dried blood.

Considering first the results of ammonium sulfate, we find that at every sampling greater nitrifying power was shown in the soil under the two-year rotation than in that under the continuous corn, and still greater power in the soil under the three-year rotation. The differences were very pronounced at every sampling the greatest being found at the fourth date when 8.086 mgs, N., 11.789 mgs. N., and 19.419 mgs. N., were formed respectively from plots 601, 602, and 604. Again in these experiments, the results from the green manured plots were very irregular. The low moisture content of the plot where clover was used as a green manure, decreased the nitrifying power of the soil at the first and second samplings, just as it decreased the total number of organisms in the soil and its ammonifying power. At the third and fourth samplings greater nitrifying power was found, it being in both cases less than that of the soil under the three-year rotation but more than

TABLE IX.

NITRIFICATION IN SOILS I.

Plot No.	Lab. No.	Addition	Nitrate mgs. N.	Average mgs. N.	Increase over untreated portions. mgs. N.
601	93	100 mgs. $(NH_4)_2SO_4$	9.996		
	94	100 mgs. $(NH_4)_2SO_4$	9.782	9.889	5.019
	95	200 mgs. D. B.	17.500		
	96	200 mgs. D. B.	17.125	17.312	12.442
602	97	100 mgs. $(NH_4)_2SO_4$	12.766		
	98	100 mgs. $(NH_4)_2SO_4$	13.125	12.945	8.075
	99	200 mgs. D. B.	20.006		
	100	200 mgs. D. B.	20.126	20.066	15.196
604	101	100 mgs. $(NH_4)_2SO_4$	17.000		
	102	100 mgs. $(NH_4)_2SO_4$	18.000	17.500	12.630
	103	200 mgs. D. B.	25.333		
	104	200 mgs. D. B.	25.960	25.646	20.776
607	105	100 mgs. $(NH_4)_2SO_4$	12.000		
	106	100 mgs. $(NH_4)_2SO_4$	11.872	11.936	7.066
	107	200 mgs. D. B.	19.762		
	108	200 mgs. D. B.	20.135	19.948	15.078
609	109	100 mgs. $(NH_4)_2SO_4$	16.816		
	110	100 mgs. $(NH_4)_2SO_4$	16.741	16.778	11.908
	111	200 mgs. D. B.	24.000		
	112	200 mgs. D. B.	23.337	23.668	18.798
901	113	100 mgs. $(NH_4)_2SO_4$	11.667		
	114	100 mgs. $(NH_4)_2SO_4$	11.521	11.594	6.724
	115	200 mgs. D. B.	19.122		
	116	200 mgs. D. B.	18.543	18.832	13.962
903	117	100 mgs. $(NH_4)_2SO_4$	9.872		
	118	100 mgs. $(NH_4)_2SO_4$	9.500	9.686	4.816
	119	200 mgs. D. B.	16.800		
	120	200 mgs. D. B.	16.972	16.886	12.016
904	121	100 mgs. $(NH_4)_2SO_4$	11.000		
	122	100 mgs. $(NH_4)_2SO_4$	11.289	11.144	6.274
	123	200 mgs. D. B.	20.000		
	124	200 mgs. D. B.	19.568	19.784	14.914
	A	Nothing	4.528		
	B	Nothing	5.212	4.870	

that of the soil under the two-year rotation. The nitrifying power of the soil where cowpeas were employed as a green manure varied at the different samplings in the exactly opposite direction. At the first two dates, it was greater than the nitrifying power of the soil under the two-year rotation, but less than that of the soil under the three year rotation. At the third and fourth samplings, it was less than the power of the soil under the two-year rotation. The drought occurring between the second and third samplings undoubtedly changed the species relationships so that there was established a different relative nitrifying power for the differences in moisture conditions would be insufficient to account for the changed relations. The nitrifying power of the soil under rye as a

TABLE X.

NITRIFICATION IN SOILS II.

Plot No.	Lab. No.	Addition	Nitrate mgs. N.	Average mgs. N.	Increase over untreated portions. mgs. N.
601	393	100 mgs. $(NH_4)_2SO_4$	28.169		
	394	100 mgs. $(NH_4)_2SO_4$	28.000	28.084	17.577
	395	200 mgs. D. B.	30.088		
	396	200 mgs. D. B.	30.693	30.390	19.883
602	397	100 mgs. $(NH_4)_2SO_4$	32.389		
	398	100 mgs. $(NH_4)_2SO_4$	31.876	32.132	21.625
	399	200 mgs. D. B.	33.541		
	400	200 mgs. D. B.	34.096	33.818	23.311
604	401	100 mgs. $(NH_4)_2SO_4$	35.170		
	402	100 mgs. $(NH_4)_2SO_4$	34.879	35.024	24.517
	403	200 mgs. D. B.	37.333		
	404	200 mgs. D. B.	37.856	37.594	27.087
607	405	100 mgs. $(NH_4)_2SO_4$	32.000		
	406	100 mgs. $(NH_4)_2SO_4$	31.970	31.985	21.477
	407	200 mgs. D. B.	33.800		
	408	200 mgs. D. B.	32.982	33.391	22.884
609	409	100 mgs. $(NH_4)_2SO_4$	33.589		
	410	100 mgs. $(NH_4)_2SO_4$	33.382	33.485	22.978
	411	200 mgs. D. B.	35.571		
	412	200 mgs. D. B.	35.896	35.733	25.226
901	413	100 mgs. $(NH_4)_2SO_4$	31.429		
	414	100 mgs. $(NH_4)_2SO_4$	32.540	31.984	21.477
	415	200 mgs. D. B.	31.520		
	416	200 mgs. D. B.	30.921	31.220	20.713
903	417	100 mgs. $(NH_4)_2SO_4$	25.000		
	418	100 mgs. $(NH_4)_2SO_4$	24.664	24.832	14.325
	419	200 mgs. D. B.	28.896		
	420	200 mgs. D. B.	28.432	28.664	18.157
904	421	100 mgs. $(NH_4)_2SO_4$	30.998		
	422	100 mgs. $(NH_4)_2SO_4$	30.841	30.919	20.412
	423	200 mgs. D. B.	30.662		
	424	200 mgs. D. B.	30.327	30.494	19.987
	A	Nothing	10.976		
	B	Nothing	10.038	10.507	

green manure was lower than that of the soil under the regular two-year rotation at every sampling, but greater than the power of the soil under continuous corn. Continuous cropping to clover showed a smaller nitrifying power in the soil than continuous cropping to corn in every case, and this was undoubtedly due partly to the difference in moisture conditions and in the treatment of the soil and partly to the different crops grown.

The results of the four-year rotation plot were irregular, also, at the first samplings low moisture conditions reducing the nitrifying power of the soil below that of the two-year rotation plot, and at the last two dates, when the moisture content was nearer that of the other soils, a greater nitrifying power than that of the soil under the two-year rotation was observed.

TABLE XI.

NITRIFICATION IN SOILS III.

Plot No.	Lab. No.	Addition	Nitrate mgs. N.	Average mgs. N.	Increase over untreated portions. mgs. N.
601	693	100 mgs. $(NH_4)_2SO_4$	14.123		
	694	100 mgs. $(NH_4)_2SO_4$	14.386	14.254	7.565
	695	200 mgs. D. B.	18.668		
	696	200 mgs. D. B.	18.439	18.553	11.864
602	697	100 mgs. $(NH_4)_2SO_4$	16.455		
	698	100 mgs. $(NH_4)_2SO_4$	16.499	16.477	9.788
	699	200 mgs. D. B.	21.499		
	700	200 mgs. D. B.	21.137	21.318	14.629
604	701	100 mgs. $(NH_4)_2SO_4$	19.921		
	702	100 mgs. $(NH_4)_2SO_4$	19.264	19.592	12.903
	703	200 mgs. D. B.	25.000		
	704	200 mgs. D. B.	24.725	24.862	18.173
607	705	100 mgs. $(NH_4)_2SO_4$	17.894		
	706	100 mgs. $(NH_4)_2SO_4$	18.199	18.046	11.357
	707	200 mgs. D. B.	23.333		
	708	200 mgs. D. B.	22.865	23.099	16.410
609	709	100 mgs. $(NH_4)_2SO_4$	15.821		
	710	100 mgs. $(NH_4)_2SO_4$	15.759	15.790	9.101
	711	200 mgs. D. B.	19.929		
	712	200 mgs. D. B.	20.356	20.142	13.453
901	713	100 mgs. $(NH_4)_2SO_4$	15.110		
	714	100 mgs. $(NH_4)_2SO_4$	14.889	14.999	8.310
	715	200 mgs. D. B.	19.120		
	716	200 mgs. D. B.	19.681	19.400	12.711
903	717	100 mgs. $(NH_4)_2SO_4$	11.928		
	718	100 mgs. $(NH_4)_2SO_4$	12.293	12.110	5.421
	719	200 mgs. D. B.	17.118		
	720	200 mgs. D. B.	16.790	16.954	10.265
	721	100 mgs. $(NH_4)_2SO_4$	16.825		
904	722	100 mgs. $(NH_4)_2SO_4$	17.136	16.980	10.291
	723	200 mgs. D. B.	22.949		
	724	200 mgs. D. B.	22.368	22.658	15.969
	A	Nothing	6.792		
	B	Nothing	6.586	6.689	

TABLE XII.

NITRIFICATION IN SOILS IV.

Plot No.	Lab. No.	Addition	Nitrate mgs. N.	Average mgs. N.	Increase over untreated portions. mgs. N.
601	833	100 mgs. $(NH_4)_2SO_4$	14.000		
	834	100 mgs. $(NH_4)_2SO_4$	13.972	13.986	8.086
	835	200 mgs. D. B.	19.833		
	836	200 mgs. D. B.	19.561	19.697	13.797
602	837	100 mgs. $(NH_4)_2SO_4$	17.500		
	838	100 mgs. $(NH_4)_2SO_4$	17.878	17.689	11.789
	839	200 mgs. D. B.	23.333		
	840	200 mgs. D. B.	23.333	23.333	17.433
604	841	100 mgs. $(NH_4)_2SO_4$	25.000		
	842	100 mgs. $(NH_4)_2SO_4$	25.638	25.319	19.419
	843	200 mgs. D. B.	29.864		
	844	200 mgs. D. B.	30.000	29.932	24.032
607	845	100 mgs. $(NH_4)_2SO_4$	19.862		
	846	100 mgs. $(NH_4)_2SO_4$	19.437	19.649	13.749
	847	200 mgs. D. B.	28.700		
	848	200 mgs. D. B.	27.522	28.111	22.211
609	849	100 mgs. $(NH_4)_2SO_4$	16.452		
	850	100 mgs. $(NH_4)_2SO_4$	16.589	16.520	10.620
	851	200 mgs. D. B.	20.662		
	852	200 mgs. D. B.	21.234	20.948	15.048
901	853	100 mgs. $(NH_4)_2SO_4$	15.555		
	854	100 mgs. $(NH_4)_2SO_4$	15.555	15.555	9.655
	855	200 mgs. D. B.	19.828		
	856	200 mgs. D. B.	20.000	19.914	14.014
903	857	100 mgs. $(NH_4)_2SO_4$	13.562		
	858	100 mgs. $(NH_4)_2SO_4$	12.981	13.271	7.371
	859	200 mgs. D. B.	18.792		
	860	200 mgs. D. B.	19.025	18.908	13.008
904	861	100 mgs. $(NH_4)_2SO_4$	18.662		
	862	100 mgs. $(NH_4)_2SO_4$	18.421	18.541	12.641
	863	200 mgs. D. B.	24.876		
	864	200 mgs. D. B.	25.194	25.035	19.135
	A	Nothing	6.000		
	B	Nothing	5.800	5.900	

TABLE XIII.

THE NITRIFICATION OF AMMONIUM SULFATE.

Plot No.	June 26. I. mgs. N.	July 8. II. mgs. N.	Sept. 16. III. mgs. N.	Oct. 25. IV. mgs. N.
601	5.019	17.577	7.565	8.086
602	8.075	21.625	9.788	11.789
604	12.630	24.517	12.903	19.419
607	7.066	21.477	11.357	13.749
609	11.908	22.978	9.101	10.620
901	6.724	21.477	8.310	9.655
903	4.816	14.325	5.421	7.371
904	6.274	20.412	10.291	12.641

Again, the variations in the results obtained at the different samplings may be attributed to the drought between the second and third dates and the consequent change in species relationships; and there is also an additional fact to be mentioned here, and that is that the effects of a drought on the bacterial flora of a soil may be quite dissimilar when different crops are being grown.

RESULTS WITH DRIED BLOOD.

Turning now to the results with the use of dried blood, we find that the agreement with the results when ammonium sulfate was used, are very good. There is the same increased nitrifying power of the soils under the two and three-year rotations over that of the soil under the continuous corn, to be noticed. Furthermore, the differences were also more pronounced here at the fourth sampling when 13.797 mgs. N., 17.433 mgs. N., and 24.032 mgs. N., were found respectively in plots 601, 602, and 604. A larger increase was again observed here for the three-year rotation over the two, than for the two-year rotation over the continuous cropping. The irregularity of the results from the green manured plots was similar to that found when ammonium sulfate was used. Low moisture conditions in the plot where clover was turned under, at the first two samplings, reduced the nitrifying power of the soil below that of the soil under the regular two-year rotation. At the last two dates, the nitrifying power of the soil, again was greater than that of the soil under the two-year rotation. In these results, too, the nitrifying power of the soil under cowpeas as a green manure was different at the first two samplings from that of the last two dates. At the former times it was more than that of the soil under the two-year rotation and at the latter it was less. The nitrifying power of the soil with rye as a green manure was less in every case than that of the soil under the two-year rotation, just as was observed in the tests with ammonium sulfate. Low nitrifying power of the

TABLE XIV.
THE NITRIFICATION OF DRIED BLOOD.

Plot No.	June 26. I. mgs. N.	July 8. II. mgs. N.	Sept. 16. III. mgs. N.	Oct. 25. IV. mgs. N.
601	12.442	19.883	11.864	13.797
602	15.196	23.311	14.629	17.433
604	20.776	27.087	18.173	24.032
607	15.078	22.884	16.410	22.211
609	18.798	25.226	13.453	15.048
901	13.962	20.713	12.711	14.014
903	12.016	18.157	10.265	13.008
904	14.914	19.987	15.969	19.135

soil under continuous clover was found at every sampling, lower than that of the soil under continuous corn, and the difference in moisture content, it must be remembered, was not great enough to account for the fact. At the first and second samplings, the nitrifying power of the soil under the four-year rotation was less than that of the soil under the two-year rotation and at the last two dates, it was greater. Here evidently the effects of the drought are clearly shown.

In general then, it may be said that the nitrification of the dried blood and of ammonium sulfate proceed parallel for the differences observed were similar in every case.

EFFECTS OF DIFFERENT CROPS.

The results of the nitrification tests as a whole, then, show that the soil under the two-year rotation possessed greater nitrifying power than that under the continuous corn and a smaller power than that under the three-year rotation. The effects of the introduction of the green manures, clover, cowpeas, and rye, were very irregular and the differences obtained in most cases were rather small, so that conclusions are difficult. It may be said, however, that the results show that rye used as a green manure in the two-year rotation did not increase the nitrifying power of the soil as much as did the ordinary two-year rotation, and this may have been due to the limiting action of moisture or to the introduction of some substances inimical to the nitrifying bacteria. Continuous cropping to clover reduced the nitrifying power of the soil below that of the continuous corn plot, and this was evidently due to the different crop and also to the difference in treatment of, and preparation for, the crop. While the results from the four-year rotation plot were irregular, due largely to the moisture conditions, there is an indication from their consideration that the effects of the crop present on the soil obscured the effects of previous cropping, due probably largely to the different methods of treatment for the crop and also to the character of the crop itself.

The effects of the different systems of cropping on the nitrifying power of the soils is shown very clearly in these results, and it is interesting to note that they are similar to the effects noted on the ammonifying power of the soils. The determination of the ammonifying power of soils may, therefore, be a measure of the power of the soils to produce nitrates, and if increased ammonifying power is observed in a soil it is an indication of the power of the soil to produce more nitrates for plant growth.

Here again it may be suggested that the benefits on the nitrifying power of the soil by the rotation of crops may be due to the more equal utilization of the different plant food constituents and the consequent avoidance of a scarcity of one constituent, or it may be due to the limiting action of certain organic substances produced in the growth of the plants, which reduce the activities of the nitrifying as well as of the ammonifying species in the soil. It may be noted here, too, that the introduction of large quantities of organic matter as green manure in the two-year rotation did not increase the nitrifying power of the soil as much as the use of a longer rotation, and the same causes may be suggested for this action as have been mentioned before. Either it was a question of moisture conditions or of the introduction of organic matter in such large amounts, or perhaps of the prevention of the disposal of the organic substances produced in the growth of the plants.

CONCLUSIONS OF NITRIFICATION TESTS.

The conclusions from these nitrification tests are:

1. The rotation of crops brought about a greater nitrifying power in soils than continuous cropping either to corn or to clover.
2. A soil under a three-year rotation of corn, oats, and clover, possessed a greater nitrifying power than one under a two-year rotation of corn and oats, or one under a two-year rotation in which clover, cowpeas, or rye were used as a green manure.
3. The use of such green manures did not always increase the nitrifying power of the soil, possibly due to moisture conditions or perhaps due to the organic matter introduced.
4. There was an indication here that the influence of the crop present on the soil obscured the influence of previous cropping.
5. The nitrification of ammonium sulfate and of dried blood proceeded almost parallel.
6. Nitrification and ammonification proceeded in the same direction.

THE NITROGEN-FIXATION EXPERIMENTS.

The results of the nitrogen fixation experiments may be found in Tables XV, XVI, XVII and XVIII. It may be seen that at the four samplings, the two-year rotation increased the nitrogen-fixing power of the soil beyond that of the continuous corn plot and the three-year rotation gave a still greater increase. These gains were quite pronounced at every sampling, the greatest difference being noted at the second date when 3.93 mgs. N., 15.07 mgs. N., and 18.25 mgs. N., fixed were the amounts recorded.

The low nitrogen fixing power of the continuous clover plot is another fact which is clearly shown. At every date the nitrogen fixing power of the soil from this plot was less than that of the soil from the continuous corn plot. We may explain this at

TABLE XV.

NITROGEN FIXATION EXPERIMENTS I.

Plot No.	Lab. No.	Nitrogen present at beginning. mgs.	Nitrogen present at end. mgs.	Nitrogen fixed. mgs.	Average nitrogen fixed. mgs.
601	125	275.28	283.19	7.91	
	126	275.28	286.38	11.10	9.50
602	127	275.28	294.33	19.05	
	128	275.28	291.15	15.87	17.46
604	129	275.28	295.92	20.64	
	130	275.28	295.92	20.64	20.64
607	131	275.28	291.15	15.87	
	132	275.28	287.95	12.67	14.27
609	133	275.28	294.33	19.05	
	134	275.28	292.74	17.46	18.25
901	135	275.28	291.15	15.87	
	136	275.28	287.95	12.67	14.27
903	137	275.28	278.42	3.14	
	138	275.28	280.01	4.73	3.93
904	139	275.28	286.38	11.10	
	140	275.28	291.15	15.87	13.48

TABLE XVI.

NITROGEN-FIXATION EXPERIMENTS II.

Plot No.	Lab. No.	Nitrogen present at beginning. mgs.	Nitrogen present at end. mgs.	Nitrogen fixed. mgs.	Average nitrogen fixed. mgs.
601	425	275.28	280.01	4.73	
	426	275.28	278.42	3.14	3.93
602	427	275.28	291.15	15.87	
	428	275.28	289.56	14.28	15.07
604	429	275.28	292.74	17.46	
	430	275.28	294.33	19.05	18.25
607	431	275.28	294.33	19.05	
	432	275.28	291.15	15.87	17.46
609	433	275.28	291.15	15.87	
	434	275.28	291.15	15.87	15.87
901	435	275.28	287.95	12.67	
	436	275.28	286.38	11.10	11.88
903	437	275.28	278.42	3.14	
	438	275.28	276.83	1.55	2.34
904	439	275.28	283.19	7.91	
	440	275.28	284.78	9.50	8.70

least in part when we recall that nitrogen fixation is so closely dependent on aeration and that factor was naturally restricted in the continuous clover plot while it was increased in the continuous corn plot. Of course the moisture conditions were also

TABLE XVII.

NITROGEN-FIXATION EXPERIMENTS III.

Plot No.	Lab. No.	Nitrogen present at beginning. mgs.	Nitrogen present at end. mgs.	Nitrogen fixed. mgs.	Average nitrogen fixed. mgs.
601	725	275.28	288.00	12.72	
	726	275.28	289.60	14.32	13.52
602	727	275.28	294.40	19.12	
	728	275.28	296.00	20.72	19.92
604	729	275.28	299.20	23.92	
	730	275.28	297.60	22.32	23.12
607	731	275.28	296.00	20.72	
	732	275.28	296.00	20.72	20.72
609	733	275.28	294.40	19.12	
	734	275.28	292.80	17.52	18.32
901	735	275.28	292.80	17.52	
	736	275.28	291.20	15.92	16.72
903	737	275.28	286.40	11.12	
	738	275.28	283.20	7.92	9.52
904	739	275.28	289.60	14.32	
	740	275.28	291.20	15.92	15.12

TABLE XVIII.

NITROGEN-FIXATION EXPERIMENTS IV.

No. Plot	Lab. No.	Nitrogen present at beginning. mgs.	Nitrogen present at end. mgs.	Nitrogen fixed. mgs.	Average nitrogen fixed. mgs.
601	865	275.28	286.40	11.12	
	866	275.28	284.80	9.52	10.32
602	867	275.28	292.80	17.52	
	868	275.28	292.80	17.52	17.52
604	869	275.28	296.00	20.72	
	870	275.28	296.00	20.72	20.72
607	871	275.28	292.80	17.52	
	872	275.28	294.40	19.12	18.32
609	873	275.28	291.20	15.92	
	874	275.28	292.80	17.52	16.72
901	875	275.28	291.20	15.92	
	876	275.28	289.60	14.32	15.12
903	877	275.28	281.60	6.32	
	878	275.28	283.20	7.92	7.12
904	879	275.28	291.20	15.92	
	880	275.28	292.80	17.52	16.72

materially affected by the difference in treatment of the soil, but they were not low in the continuous clover plot at every date, and still the nitrogen fixing power remained small.

In the case of the green manured plots, the results are very difficult to interpret. Where clover was turned under, at the first date the nitrogen fixing power of the soil was less than that of the soil under an ordinary two-year rotation, but the moisture content of the soil at that date was abnormally low which would largely account for the difference. At the other samplings, while the moisture content was still somewhat smaller than that of plot 602, the nitrogen fixing power of the soil was greater than that of the soil under the two-year rotation, but less than that of the three-year rotation plot. Where cowpeas were employed, at the first two dates, slightly greater nitrogen fixing power was evidenced than that of the two-year rotation soil but less than that of the soil under the three-year rotation, while at the last two samplings the nitrogen fixing power of the soil was less than that of the two-year rotation soil. When rye was turned under the nitrogen fixing power of the soil was less than that of the two-year rotation plot in every case.

The results are somewhat surprising, to say the least, when we remember that the nitrogen fixing power of a soil depends very largely on the presence of sufficient carbonaceous material to supply the energy necessary for the fixation of the nitrogen. It might be supposed that where large amounts of carbonaceous substances were added, greater nitrogen fixing power would be occasioned, but such was not the case and in fact with the rye there was a depression in nitrogen fixing power. It is possible that the materials introduced were not of use to the

TABLE XIX.

NITROGEN-FIXATION EXPERIMENTS.

(SUMMARIZED)

Plot No.	June 26. Nitrogen Fixed. I. Mgs.	July 8. Nitrogen Fixed. II. Mgs.	Sept 16. Nitrogen Fixed. III. Mgs.	Oct. 25. Nitrogen Fixed. IV. Mgs.
601	9.50	3.93	13.52	10.32
602	17.46	15.07	19.92	17.52
604	20.64	18.25	23.12	20.72
607	14.27	17.46	20.72	18.32
609	18.25	15.87	18.32	16.72
901	14.27	11.88	16.72	15.12
903	3.93	2.34	9.52	7.12
904	13.48	8.70	15.12	16.72

nitrogen fixing species or they might have favored some other species which over grew the nitrogen fixers. In the case of the four-year rotation, the nitrogen fixing power of the soil was less than that of the two-year rotation soil, but the difference in crop probably would account largely for the difference in fixing power. Furthermore the differences in moisture and in treatment of the soil for the different crops would also affect materially the nitrogen fixing power of the soils.

It is certain then from these results, that not only was the nitrogen fixing power of the soil affected by the crop grown, but it was also materially altered by the rotation of crops which was practiced.

The cause for these effects of the rotation of crops may again be sought either in the removal by certain crops of some constituents needed by the nitrogen fixing species or in the production by some crops of substances which inhibit the activity of the organisms and the neutralization or destruction of such substances by the growth of some other crop, or in the lengthened period between two succeeding crops of the same plant, allowing opportunity for the destruction of the toxic substances by bacterial or by chemical action.

CONCLUSIONS OF NITROGEN-FIXATION TESTS.

The conclusions which may be drawn from these experiments are similar to those obtained in the other work:

1. The rotation of crops caused the development of greater nitrogen fixing power in the soil than the continuous cropping to corn or to clover.
2. A soil under a three-year rotation of corn, oats, and clover, possessed greater nitrogen fixing power than one under a two-year rotation of corn and oats, or one under a two-year rotation in which clover, cowpeas, or rye were turned under.
3. The use of a green manure in the two-year rotation did not always increase the nitrogen fixing power of the soil, possibly due to unfavorable moisture or aeration conditions, or to the inhibiting action of the organic matter on the nitrogen fixing species.
4. There was an indication that the influence on the nitrogen fixing power of the soil of the crop present on the soil was greater than that of the previous cropping, and this might have been due to the crop itself or to the treatment of and preparaion for, the crop.

THE CROP YIELDS.

Table XX contains the crop yields for 1911 on the plots used in this experiment, and it is interesting to note some agreements with the results of the bacteriological tests. Plot 601 produced 35.5 bushels of corn per acre; plot 602, 46.0 bushels; and plot 604, 50.7 bushels per acre. Here the greater crops of corn on the plots under rotation are clearly shown, the two-year rotation plot giving a larger yield than the continuous corn plot and the three-year rotation increasing the yield still further. Plot 607 gave a yield of 52.7 bushels of corn per acre, a slightly larger amount than that given by the three-year rotation, and plots 609 and 901 both gave smaller yields than plot 602, 32.5 bushels and 43.2 bushels per acre being the yields obtained. The yields from plots 903 and 904 are, of course, not comparable, 1.56 T. of clover and 1.89 T. of clover per acre being obtained, showing merely the increase in the clover crop on the plot under the four year rotation over that on the continuous clover plot.

TABLE XX.

THE CROP YIELDS.

Plot No.	Yield per acre (1911)
	Corn.
601	35.5 bu.
602	46.0 bu.
604	50.7 bu.
607	52.7 bu.
609	32.5 bu
901	43.2 bu.
	Clover.
903	1.56 tons.
904	1.89 tons.

Turning now to the bacteriological results, we find that the total number of organisms, the ammonifying power, the nitrifying power, and the nitrogen-fixing power of the soils under the three year rotation were greater than under the two-year rotation, and those under the two-year rotation were greater than on the continuous corn plot. The correspondence here with the crop results is very striking.

All the bacteriological tests of plot 607 where clover was plowed under as a green manure showed slightly smaller bacterial activities than in the three-year rotation plot, but in most cases the differences were very small and the gain in crop yield which was found here was likewise small, so that the differences are hardly definite enough to permit of conclusions being drawn. In the plots where cowpeas and rye were used as green manures, the crop yields were less than those on the two and three year rotation plots. The bacteriological tests all showed less bacterial activity in these plots than in the three year rotation plot, but not in all cases were they less than in the two-year rotation plot.

The variations in the bacteriological results of these plots have been discussed and the need for further confirmatory data with regard to them has been stated. It may be mentioned, however, that in almost every case the bacterial activities in the plot with the rye turned under were less than in the two year rotation plot. As a rule, therefore, the results from the green manured plots were not definite enough to permit of general conclusions but there are indications that the crop yields and bacterial activities bear some relation to each other and as was stated earlier in this work further study is being planned along this line to check these results and add if possible some additional facts.

The bacteriological tests of the plots in clover showed in every case much greater bacterial activities in the four-year rotation plot than in the continuous clover plot, and the crop yield from the former plot was greater than that from the latter plot. Here again, therefore, bacterial activities and crop production seem to proceed in the same direction.

While, therefore, definite conclusions must not be drawn from this one season's work, it may be said that there is evidence from these results that bacterial activities and crop production run parallel; in other words, they lend support to the theory that soil treatment which increases bacterial activities in the soil should increase the crop-producing power of the soil. Furthermore, there is furnished support for the assumption that laboratory tests of soils for bacterial activities indicate the relative fertility of the soils.